

ON the MATURATION of the PELAGIC OVUM
of TELEOSTEANS, and its RELATION
to CERTAIN PHENOMENA in the LIFE-
HISTORY of the SPECIES

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I. I N T R O D U C T I O N

Precise knowledge respecting the mature eggs of the great majority of marine osseous fishes is of comparatively recent date. The structure or anatomical relations of the reproductive organs were early made the subject of investigations by such authorities as Rathke (65) Cuvier and Valenciennes (20) Vogt (3) Owen (57) Hyrtl (44) and Waldeyer (79) as well as by other zoologists; and the eggs, embryology and development of several species were studied before 1866 by Rathke (66) von Baer (78) Rusconi (68) Vogt (77) Doyère (23) Joh. Müller (53) Reichert (67) Bruch (13) Lereboullet (14, 15) Stricker (74) Steenstrup (73) Ransom (62) and others. Since the year named a large number of investigations have been made by various naturalists, especially in connection with the embryology of the group, many of which will be referred to in the course of this Thesis. Previous to the period indicated the researches on teleostean embryology were confined either to fresh-water species, or / -

or to one or two marine shore forms, in which the eggs are carried by one of the parents until they hatch, namely the pipe fishes (Syngnathus) where the male performs the function of nurse, and the viviparous blenny (Zoarces viviparus) in which the embryonic development takes place within the ovary, the young fishes being retained until they are nearly two inches in length. Thus, the researches of von Baer were confined to the perch, and to several species of Cyprinoids; those of Rusconi also to the perch, carps and pike; those of Lereboullet to the pike, perch, trout and salmon; those of Vogt to Coregonus palæa. The other species of Teleosteans whose eggs or embryology formed the subject of investigation by the zoologists referred to (previous to the period named) were the Sticklebacks (G. leiurus, G. pungitius) the Ruffe (Acerina vulgaris) the river-bullhead (Cottus gobio) the chub (Leuciscus^c cephalus) the viviparous blenny (Zoarces viviparus) and the snake pipe-fish (Syngnathus ophidium); the two latter being the only representatives of the marine Teleosteans.

One reason why the earlier investigations were made /

made almost exclusively on fresh-water forms was, no doubt, the comparative ease with which living eggs could be obtained, the relatively large size of the eggs, and the convenience with which they could be preserved in fresh-water in the living condition. It is also obvious that from the fact stated above, that the fecundated eggs are carried by one of the parents in Zoarces and Syngnathus, it was not difficult to procure for study specimens in which the embryonic development was at different stages; although the development of Zoarces presents special and important features in itself. But there was another reason, inasmuch as previous to the time named very little was known about the eggs or oviposition of marine Teleosteans. It was not until the discoveries of Sars, to be presently referred to, respecting the eggs and spawning of the cod, haddock, and mackerel, that the attention of zoologists was specially directed to the importance of the subject. Since that time, although researches have naturally continued to be made on fresh-water forms, by far the greater number have been made on marine species, a circumstance also due to the establishment of marine laboratories in /

in various countries, which have provided facilities for the work that did not previously exist.

In 1864 G.O.Sars, in the course of an investigation into the cod fisheries of the Lofoden Isles, Norway, discovered that the mature eggs of the cod consisted of minute, perfectly transparent, crystalline spheres, which floated about separately in the surface waters of the sea (7.70 Indberetning for 1864, p.17). In the following year he artificially fecundated the eggs of the cod and studied the development of the embryo; and he showed that the animal pole was inferior, and not superior, as is the rule in demersal eggs. He also found in the same year that the eggs of the haddock and the mackerel were buoyant like those of the cod, as well as the eggs of some other species which he succeeded in hatching, but not in identifying. These discoveries of Sars were of great importance, both from the scientific and the practical point of view. They proved that the eggs of Teleosteans might float freely and separately at the surface, instead of lying, as was previously believed to be invariably the case, on the bottom, and they /

they opened up a wide field for further investigations. In 1867 A.W.Malm (52) studied the eggs of the flounder, which were found to be buoyant; in the following year Kupffer (46) published a paper on the development of osseous fishes, dealing principally with marine forms (Gasterosteus spinachia, Gobius minatus, G. niger). In 1874 Hæckel (34) and E.van Beneden (75) studied the development of unidentified pelagic fish eggs in the Mediterranean; and from about that time, and especially during the last fifteen years, a large number of memoirs and papers dealing with the nature of the eggs and the embryology of teleostean fishes have been published, many of which are appended in the Bibliographical List at the end of this Thesis (p. —). Most of these memoirs deal chiefly with the diagnostic description of the mature and fertilised eggs or with the embryological development; comparatively few include the intra-ovarian development.

The researches that have been made since Sars' discovery of the floating eggs of the cod in 1864, have /

have shown that what he at the time regarded as exceptional is in reality the usual condition; that the mature ovum of marine Teleosteans is in the majority of cases buoyant, or pelagic, and that those species whose eggs are demersal - lying at or attached to the bottom or to solid objects - are shore-forms, which, with the exception of the herring, are of little economic importance. All the Gadoids (except Lota vulgaris, which is a fresh-water form) and European flat fishes have pelagic eggs, as have also the mackerel, pilchard, anchovy, sprat, the gurnards, and many others. On the other hand, none of the species which spawn in fresh-water have pelagic eggs, for reasons which will be evident later.

The investigations described in this paper deal mainly with the later stages of the intra-ovarian growth of the teleostean ovum, especially with the maturation of the pelagic ovum, and with certain phenomena in the embryonic, larval, and adult life of the species, which are connected with or dependent upon the changes which occur at the period of maturation.

The most important point which I have been able to /

to determine is that the final changes in the maturation of the pelagic ovum, while still within the ovary, is accompanied by a comparatively sudden and relatively great accession of fluid from without, which dissolves the yoke spherules, is associated with the disappearance of the germinal vesicle and the increase of the cortical layer of germinal matter; distends the ovum to several times its former volume, thinning the zona radiata to a corresponding extent, renders it of crystalline transparency, and reduces its specific gravity so that it floats in sea water of ordinary density. The process is quite different from the gradual growth of the ovum within the follicle with which it has been confounded. It is a rapid physical change, which is essential to enable the egg to float, and to become therefore a pelagic egg. The dilution of the nutritive yolk by several times its volume of fluid explains many phenomena hitherto obscure in the development of the embryo of pelagic eggs, such as the comparatively brief duration of the period of development, the absence of vitelline circulation, the rapidity with which the cytoplasm /

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deutoplasm is absorbed in the larval stage after the embryo issues from the egg, and the relatively small size and ill-developed condition of the embryo when it is hatched. Physiologically, so far as concerns its nutritive power, the mature demersal egg (in which this marked and sudden transformation of the yolk^l does not occur) is comparable not to the mature pelagic egg, but to the preceding opaque stage. The great and sudden increase in the volume of the ovum before it is expelled from the ovary, also explains a number of phenomena associated with the spawning or oviposition and the sexual relations of the adults, which are described below.

That the fully mature pelagic ovum of Teleosts is translucent and larger than the opaque eggs present at the same time in the ovary has long been known, but I have been unable to find in any of the numerous papers dealing with the subject any indication of the real change which occurs. As will be shown later, the descriptions given by various authors give no hint that the final change is due to a special cause, while they agree with the view that it is /

is merely a continuance of intra-ovarian growth. The reason that this stage has hitherto escaped close attention is most probably owing to the fact that the few who have concerned themselves with intra-ovarian pelagic eggs, have almost exclusively studied only sections of the ovary showing the immature or opaque ova, while those who have dealt with the fully mature eggs have almost always been concerned with the changes following impregnation, or with their identification and morphological description; perhaps also from failure to keep clearly in mind the fact that a small increase in the diameter of a sphere represents a large increase in its volume.

The methods adopted in my investigations were as follows, (1) the examination of the immature and ripe ovaries in the living fish or in the fresh condition, without preparation, (2) the microscopical examination of parts of the fresh ovaries and of the intra-ovarian eggs at various stages, without preparation, in various media, (3) the study of stained and mounted sections of the ovaries and eggs prepared in the /

the usual way, (4) the determination in certain cases of the general nature and quantity of the intra-ovarian fluid present in ripening ovaries, (5) experimental observations on the physical properties of the opaque and the transparent mature eggs, and on some other points.

The species whose ovaries and eggs have been investigated comprise the following :

A. With Dispersed Pelagic Eggs :

Grey Gurnard	(<i>Trigla gurnardus</i>)
Red Gurnard	(<i>Trigla cuculus</i>)
Lesser Weever	(<i>Trachinus vipera</i>)
Mackerel	(<i>Scomber scomber</i>)
Dragonet	(<i>Callionymus lyra</i>)
Cod	(<i>Gadus morrhua</i>)
Haddock	(<i>Gadus aeglefinus</i>)
Bib	(<i>Gadus luscus</i>)
Whiting	(<i>Gadus merlangus</i>)
Saithe	(<i>Gadus virens</i>)
Ling	(<i>Molva vulgaris</i>)
Fourbearded Rockling	(<i>Motella cimbria</i>)
Tusk /	

Tusk	(<i>Brosmius brosme</i>)
Halibut	(<i>Hippoglossus vulgaris</i>)
Long Rough Dab	(<i>Hippoglossoides limandoides</i>)
Turbot	(<i>Rhombus maximus</i>)
Brill	(<i>Rhombus laevis</i>)
Sail-fluke	(<i>Arnoglossus megastoma</i>)
Plaice	(<i>Pleuronectes platessa</i>)
Lemon Dab	(<i>Pleuronectes microcephalus</i>)
Witch	(<i>Pleuronectes cynoglossus</i>)
Common Dab	(<i>Pleuronectes limanda</i>)
Flounder	(<i>Pleuronectes flesus</i>)
Sole	(<i>Solea vulgaris</i>)
Solenette	(<i>Solea lutea</i>)
Norwegian Topknot	(<i>Zeugopterus^uus norvegicus</i>)
Sprat	(<i>Clupea sprattus</i>)

B. With Floating Eggs imbedded in Mucus :

Angler, or Monk Fish	(<i>Lophius piscatorius</i>)
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C. With Demersal Eggs :

Short-spined Cottus	(<i>Cottus scorpius</i>)
Long-spined Cottus	(<i>Cottus bubalis</i>)
Pogge	(<i>Agonus cataphractus</i>)
Lumpsucker /	

Lumpsucker	(Cyclopterus lumpus)
Diminutive Sucker	(Liparis montagui)
Catfish	(Anarrhichas lupus)
Lesser Sand Eel	(Ammodytes tobianus)
Sparling	(Osmerus eperlanus)
Herring	(Clupea harengus)

The immature ovaries of the John Dory (Zeus faber) and the common eel (Anguilla vulgaris) were also examined. The eggs of the Angler are pelagic in the sense that they float at the surface of the sea, but they are not separate and dispersed like ordinary pelagic eggs, being imbedded in a large quantity of mucus forming a long band which floats them; the eggs themselves are of the demersal type. The eggs of the John Dory have not hitherto been described; from a study of the features of the immature eggs they appear to be demersal. The ova of the eel and conger, according to the recent researches of Grassi, (29) are pelagic, as was previously surmised by Raffaele (64). Stained and mounted sections of the ovaries and intra-ovarian eggs of the following species were prepared /

prepared and examined : - Haddock, Whiting, Bib, Grey Gurnard, Plaice, Lemon Sole, Flounder, Common Dab, Witch, Sole, Solenette, Norwegian Topknot, Long Rough Dab, John Dory, Viviparous blenny, Monk fish, and Common Eel. The eggs and portions of the ovaries of the following species were examined microscopically in the living or fresh condition in the ovarian succus, and in various media : - Cod, Haddock, Whiting, Ling, Saithe, Bib, Fourbearded Rockling, Gurnard, Red Gurnard, Turbot, Brill, Halibut, Plaice, Lemon Sole, Witch, Common Dab, Flounder, Sole, Solenette, Norwegian Topknot, John Dory, Lumpsucker, Catfish, Herring.

The quantity and general character of the ovarian fluid was determined in the cod, ling, haddock, flounder, and plaice; by the courtesy of Professor Rutherford a special determination was made in the Physiological Department by Dr Milroy, with respect to the composition of the ovarian fluid of the plaice. Dr Milroy was also kind enough to investigate many other points of interest bearing upon my work, which are referred to later.

II. THE MATURATION of the OVUM. /

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1. The Ovaries :

The anatomy and structure of the female reproductive organs of Teleostei have been described by His (38), Brock (10), and Macleod (51) as well as by the older writers previously referred to. Such points in the minute structure as bear upon my subject will be explained later when dealing with the maturation of the intra-ovarian eggs. It is, however, necessary here to refer to some general features of the ovary in the species which produce pelagic eggs and in those whose eggs are demersal. If the ovaries of either form be examined before the advent of the spawning season, they will be found to be composed for the most part of relatively large, yolked, opaque eggs, destined to be extruded when the fish begins to spawn; but, in addition, other ^{ova}~~ova~~ are found much smaller and not opaque. Among almost all forms with demersal eggs the difference in size, as well as in appearance, between these minute eggs and the large ones is very marked; and, further, the larger eggs /

eggs approaching maturity are all of nearly equal size (26). For example, in the lumpsucker (Cyclopterus lumpus) at this period the great mass of the eggs range about 2.2 mm in diameter, while the largest of the small eggs scattered about in the stroma do not exceed 0.4 mm. It is the same with many other forms such as the catfish (Anarrhichas lupus) the herring (Clupea harengus) the smelt (Osmerus eperlanus) the salmon (Salmo salar), etc., the two sizes of eggs being sharply demarcated from one another, and the larger being subequal in size. This condition is associated with rapid oviposition, the whole of the large eggs being extruded together en masse, as in the case of the lumpsucker, or at very brief intervals, as with the herring and the smelt. In some other species with demersal eggs, such as the sticklebacks (Gasterosteus) the pipe-fishes (Syngnathus) and the diminutive suckers (Liparis) three series of eggs may be observed, each marked off from the other by difference in size, the two larger classes both containing yolk. Thus in Liparis montagui, /

montagui, at the spawning season, mature eggs practically of uniform size (1.1 to 1.2 mm) are present in the ovaries together with a series ranging from about 0.5 to 0.6 mm and a third series of minute clear yolkless ova not exceeding 0.2 mm. This condition is met with in small species in which comparatively few eggs are deposited at one time, and which shed their eggs in more than one batch or lot during the season. The successive spawnings have been specially observed among the sticklebacks (62).

In the ovaries of species which produce pelagic eggs, there is not always the same sharp demarcation between the large, yolked eggs and those that are small and transparent. Gradations in size may be detected between the two groups, but the degree in which this transition exists varies very much in different species. In the larger forms, such as the cod, ling, plaice, tusk and turbot, there are comparatively few eggs of intermediate size, while in the haddock, whiting and gurnard, the intermediate eggs are very numerous, and the gradation between the largest /

largest and the smallest is readily perceived. The latter condition is associated with a prolonged spawning period. A female gurnard or whiting takes many weeks or even two months to shed her eggs, and there is thus time for the smaller yolked eggs to grow larger and to be expelled at the close of the spawning period. In the larger forms the oviposition, although still prolonged over several weeks, owing to the changes associated with maturation, to be described later, is more rapid, the eggs shed in a given season being almost all pretty equally developed when spawning begins.

If we now compare the fully mature ovaries of a teleostean whose eggs are pelagic with those of one whose eggs are demersal, a striking difference will be perceived. In the latter, as already indicated, the eggs which are shed are developed equally; they grow gradually until they reach a certain size, which is practically uniform in a given species, and they are then expelled (Fig. 1). But in the species with pelagic eggs, examination of the mature ovary reveals /

reveals the presence of large, clear, eggs of crystalline transparency, scattered throughout its substance, presenting a marked contrast both in size and appearance to those around them, which are white, opaque and smaller (Fig. 2). These are the mature ova ready to be shed. They have burst through the follicular covering; and they gradually accumulate in the lumen of the ovary and are normally expelled in small quantities at a time by the oviduct. If a transverse section be made of such an ovary after spawning has been in progress for some time, the central portion will be found filled with the large transparent eggs, and gentle pressure on the uninjured organ causes them to issue in a stream, none of the opaque eggs escaping unless great pressure is used.

It is important to observe that extremely few eggs of intermediate size or character can be detected. I have devoted close attention to this point, and have carefully examined many thousands of eggs with this special object in view - both in the fresh /

fresh condition and after preservation. The fact can readily be demonstrated by heating a portion of the ovary in water, and then picking it to pieces; the opaque eggs become chalky white and of firmer consistence without sensible alteration in size, while the large transparent eggs exhibit a comparatively faint milky opacity and remain soft and watery. Their separation into two groups is obvious. It can also be seen in mounted sections, but not so well because of the shrinkage which usually occurs from the action of the re-agents.

The eggs of intermediate size that are occasionally to be observed - not more as a rule than one or two in a thousand - resemble much more closely the opaque eggs in size and character than those which are fully mature and transparent. They are a little larger than the opaque eggs, not transparent, but slightly translucent, and in some cases showing the position of the germinal vesicle by a lighter spot in the centre. They contain distinct yolk spherules, somewhat larger and paler than in the /

the ordinary opaque egg (Fig.3). They will be referred to later.

The difference in size between the largest opaque eggs and the mature transparent eggs is very considerable. The increase in the diameter of the ovum varies in different species; it may in some cases exceed eighty per cent; but inasmuch as the largest opaque eggs are usually, and the fully mature eggs are always spherical,^x a comparatively small increase in the diameter indicates a considerable increase in the volume. Thus the opaque eggs of the plaice, just before they undergo the hyaline enlargement, have a volume of about 0.67 cubic millimetres, while the average volume of the mature transparent egg is 3.764 cubic millimetres; obviously a very great difference. Particulars for each species are given later.

The proportional number of the transparent mature eggs present at a given time in the ovaries of any /

x The eggs of the anchovy (Engraulis encrasicolus) are peculiar among pelagic eggs in being elongated ovate (Wenkenbach,).

any species depends upon the period in relation to the beginning or termination of oviposition; but the proportion also varies to some extent according to the species. At the commencement of spawning, very few are present. The proportion increases as the spawning progresses, and towards the end the cavities of the ovaries are found to be distended exclusively, or almost exclusively, with the clear mature eggs. In almost all species in which the eggs are pelagic, the ovaries are of relatively great size. In most of the Pleuronectidae they extend posteriorly on either side of the haemal spines nearly to the root of the tail. The great distension of the abdomen in the female cod, haddock, etc., at the spawning time is familiar to all.

There is thus a striking difference in the condition of the ripe ovaries in fishes producing pelagic and in those producing demersal eggs. In the latter the mature eggs, (which generally possess a very tough, thick capsule or Zona) reach their full size by gradual growth, and are then discharged en masse, /

masse, or only with such delay as is necessary for their oviposition in the mode proper to the species, (as in a layer on shells among the Gobies, scattered clusters, etc.); while in the former, each egg reaches its full size by a rapid expansion before it is extruded, and the process affects only a small proportion of the eggs at one time, which are then discharged from the ovary, the remainder undergoing a similar change successively and being in turn discharged. The gross volume of the fully mature eggs discharged in this way by a single female during a spawning season may approximate to the total volume of the rest of the body.

Little need be said here about the ovarian blood supply. It is, of course, large, both during the growth of the eggs and during the actual spawning. Each egg is surrounded by an anastomosing network of capillaries, often beautifully displayed by the contrast between the red blood contained in them and the white opaque surface of the eggs (Fig. 2). In the spent ovary, the tissues are sanguineous, and contain /

contain extravasations and abundance of red blood corpuscles.

It is, however, necessary to speak in fuller detail of the intra-ovarian fluid.

2. The Intra-ovarian Fluid :

Little attention has hitherto been given to the free intra-ovarian fluid, which is found in Teleosteans bathing the lamellae and the mature eggs while still within the ovarian cavity. It has generally been regarded as (1) a lubricant to facilitate the escape of the eggs during the process of oviposition (2) in certain forms, as a cementing substance to bind the deposited eggs to one another, or to foreign bodies. Among attached or adherent demersal eggs, adhesion is in some cases effected by an obvious modification of the cortical part of the egg-membrane, which is formed of two layers, as among the species of Gobius and in the smelt (Osmerus). In many cases it is caused simply by a stickiness of the external surface of the zona, which is soft when the egg is extruded, and in other cases by the hardening of the fluid which bathes the eggs and is expelled with them. The distinction between the two latter cases in different species does not appear to have been carefully investigated; but the adhesion /

adhesion of the extruded demersal eggs by the hardening of the 'mucilaginous' fluid around them is described by many writers (Ransom 62, Von Baer 78, McIntosh and Prince 54, etc.). In another group the eggs are imbedded in a slimy mucoid matrix which may float them, as in Scorpaena (Hoffman 39, Raffaele 64) Fierasfer (Emery 25) and Lophius (Agassiz and Whitman 3, Prince 60). The origin of this slimy substance has not hitherto been determined. Hoffman is of opinion that it does not arise in Scorpaena from modification of the capsule of the egg; he looks upon it as probably originating in the peculiarly modified connective tissue of the theca folliculi. I have not investigated the subject in Scorpaena or Fierasfer, which are Mediterranean species, but it will be shown in this paper that the mucoid substance enveloping the eggs of Lophius is probably produced either by a layer of columnar epithelium lining the spaces between the ovigerous ingrowths, or by a secretion bathing the egg follicles (p. 51 Figs 18, 19). It may be noted here, that /

that in the viviparous blenny the young fishes are nourished by the albuminous fluid secreted by the ovary. In species producing isolated pelagic eggs, the intra-ovarian fluid is expelled with the mature eggs during the act of oviposition. It is watery, mingles with the sea water and disappears; it performs no function subsequent to its expulsion. It may readily be obtained by pressing the mature eggs gently from the female and separating the eggs by allowing the fluid to drain through muslin or fine silk netting. It is also very evident in opening a ripe ovary. The only reference I have found to this liquid - apart from the mere statement of the presence of a 'lubricating' fluid in the ovaries - is in a paper by Hensen "On the occurrence and quantity of the eggs of some Baltic fishes, especially those of the plaice, flounder and cod". (36)

Hensen, in order to determine the precise specific gravity of the mature eggs of the cod when they are expelled from the oviduct, first ascertained the specific gravity of the eggs and fluid combined, and then /

then the specific gravity of the fluid when separated from the eggs. He found the fluid from the ovaries of the cod to have a specific gravity, as determined by the ~~piez~~^{cn}ometer, of 1.01115 at a temperature of 8.7° C; he described it as alkaline, and as containing an albuminoid substance which separated on boiling, or by the addition of acetic acid, and which on precipitation by alcohol could not again be dissolved. He found the quantity of the liquid to vary greatly, but to range as a rule between fourteen and twenty per cent of the total volume of the matter discharged from the ovaries in the cod. In regard to its source, he says, "the eggs freshly shed lie in a liquid that may well be called Liquor folliculi, as it probably originates in Graaf's follicles" (p. —). Later writers who have specially dealt with the ovaries of Teleosteans either do not mention the presence of intra-ovarian fluid, or refer to it only as a lubricant (24.54:69). Neither does Hammarsten in his treatise on Physiological Chemistry (35) when dealing with female reproductive /

reproductive organs.

If the ovary of a Teleostean, whose eggs are pelagic, such as a ling, cod, plaice or halibut, be opened some time before the spawning season, it will be found to contain a very small amount of free fluid. The lumen is at this time, as a rule, for the most part occluded by the ingrowth of the ovigerous lamellae, but fluid exists in the interstices and between the opposed surfaces. I did not, unfortunately, attempt to collect the fluid at this stage, or determine its amount or nature; but the quantity is very small, not more than to keep the surfaces wet. Later when mature eggs are present and oviposition has begun, or is about to begin, the quantity of fluid is much greater. From the ovaries of a ling, which had just begun to spawn, and which (ovaries) weighed 5 lbs 6½ oz, the quantity of fluid which drained off in the course of fifteen hours was 122 cubic centimetres. From the ovaries of a cod, which had been spawning for a short time - that is to say, in which the central cavity of the ovary /

ovary was occupied by large mature eggs, the lamellae throughout its mass studded with similar eggs, and the walls of the organ somewhat flaccid - the quantity of fluid which drained off in thirteen hours was 182 cubic centimetres. The ovaries weighed 6 lbs 2 oz. From the ovaries of a saithe or coalfish, in which the great mass of the organ consisted of mature transparent eggs, and which weighed 3 lbs 2 oz, the quantity of fluid which drained off in thirteen hours was 78 cubic centimetres. The ovaries of a haddock, which had nearly completed spawning, and which were very watery-looking, yielded 15 cubic centimetres. They weighed $3\frac{3}{4}$ oz. The ovaries of a flounder, also almost spent, yielded 14 cubic centimetres; the fish weighed 1 lb 3 oz.

In each of these cases, the method adopted was to suspend the ovary over a beaker, with a cover of muslin to retain the eggs, the oviduct and a portion of the wall of the ovary being first slit. In each case the fluid was a little turbid and slightly glairy, leaving a sensible but faint stickiness on the fingers; it had a saltish taste and rendered reddened /

reddened litmus paper faint purplish-blue. It did not markedly affect turmeric paper. On heating it, a fairly copious grayish-white coagulum was formed. The specific gravity of the fluid from the ling, determined by a delicate hydrometer (Challenger pattern, No.18) was, after correction for temperature, 1,0127, and that of the cod was 1,0119.

The method described was however unsatisfactory, inasmuch as small blood vessels were cut in slitting up the tissues, and the fluid occasionally possessed a faint sanguineous tinge as it dropped from the ovary; microscopical examination also revealed the presence of a few yolk spherules, showing that some of the opaque eggs had been ruptured. I therefore adopted the method employed by Hensen of whose observations on the fluid in cod ovaries I had been previously unaware. For this purpose the ovaries of a number of spawning plaice were gently pressed, and the matter discharged, including eggs and fluid, measured 517 cubic centimetres. By allowing this to drain through silk netting, the eggs were separated from the great bulk of the fluid, which then measured /

measured 51.5 cubic centimetres; there still remained of course a considerable amount adhering to the surface of the eggs, so that this statement must not be taken as showing the precise quantitative relation. The fresh ovarian fluid of the plaice was handed to Dr Milroy, who finds that the ovarian fluid from the plaice has a specific gravity of 1.0116 as determined by the pycnometer, with a practically neutral reaction, that it is very pale yellow, almost transparent, and contains a large amount of chlorides, phosphates being absent. The amount of chloride of sodium found to be present was 0.766 grammes to each 100 cubic centimetres of the fluid; and the total of the albumins was 0.838 grammes to each 100 cubic centimetres. With regard to the latter, Dr Milroy states that a peculiarity is the absence of mucin or pseudomucin, which are usually present in ovarian fluids.

3. The Intra-ovarian Development of the Ovum :

As previously stated (p.) I have investigated the intra-ovarian development of the ova and their maturation in a number of Teleosteans, belonging for the most part to the group in which the mature egg is pelagic. The material consisted of ovaries in various stages of development, taken both from young fishes which had not attained sexual maturity, and from those in which spawning was imminent, in progress, or past. The investigation was made partly on fresh ovaries removed from the fish while it was still alive, or shortly after its death; in one or two cases the eggs were taken from the ovaries in successive portions, while the fish was kept alive in a suitable vessel supplied with sea water; part of the skin and subjacent tissues being removed for the purpose. The fresh material was examined in the intra-ovarial liquid, and also in fresh water and sea water; re-agents, consisting chiefly of solutions of common salt, acetic acid solutions, and osmic acid were employed; and a great deal was ascertained by graduated compression of the eggs. The prepared /

prepared material consisted of portions of ovaries, hardened chiefly in picrosulphuric acid, or Perengy's fluid, and spirit, and stained for the most part with borax-carmines; haematoxylin, saffranin and eosin were occasionally employed.

Compared with the numerous researches which have been made on teleostean eggs in connection with segmentation and the development of the embryo, little study has been given to their intra-ovarian development. This is more especially the case with the species producing pelagic eggs. In addition to observations made by Cunningham (16), and by a few others on forms which they were studying (Emery, 25; Bambeke 7.8) an investigation was made on a number of species belonging to the group with pelagic eggs by Scharff (72) and by Calderwood (14). Scharff's paper is short but of value; although some of his conclusions can scarcely now be upheld. He investigated the intra-ovarian development of the eggs of seven species with pelagic eggs, and principally those of the common gurnard. Calderwood examined the ovaries of eight species, his descriptions and /

and conclusions being chiefly based on what he found in the common dab and hake. It is however sometimes difficult to understand his meaning.

It has already been stated that examination of the ovary of a bony fish, whose eggs are pelagic, during the spawning season, reveals the presence of eggs in various stages of development. Observers have differed somewhat in the classification of the smaller eggs, but a convenient division of those found in such an ovary as I have described may be stated as follows : (1) the fully mature large, free, transparent eggs in which the germinal matter has separated more or less completely from the deutoplasm, and forms a cortical or perivitelⁱlⁱline layer enclosing the latter; (2) smaller, opaque eggs, still contained within the follicle, with a relatively thick egg-membrane, distinct yolk spherules and germinal vesicle; (3) small transparent eggs which exhibit a conspicuous spherical or sometimes ovate nucleus, and a mantle of protoplasm. This classification does not include the minute germ-cells, which /

which I have not studied; but it includes all those from the period when they can be distinctly recognised as possessing a nucleus and a protoplasmic mantle to the time when they are ready for extrusion and impregnation - during therefore the whole period of intra-ovarian growth.

In some cases, as has been already explained, there is no sharp demarcation between the first and the second stages, so far as concerns the size of the ovum; there is invariably a sharp demarcation between the second and the third stages, both in respect to the size and the characters of the ovum. In the whiting and gurnard, the gradations can be well traced during the spawning time, which in these species is prolonged, but in some others, as the plaice, ling, tusk, etc., the transitional forms are relatively few, nearly all of those in the second group approximating to a definite size. In these the spawning is more rapid, and time is therefore not available for the smaller yolked eggs to grow large enough to participate in the spawning process of that season. Further, examination of the ovaries after /

after the completion of spawning shows that the yolked eggs which are retained within the ovarian stroma undergo degenerative changes, and that there is a pause, so to speak, in the activity of the organ with respect to the formation of yolk. In the ovaries of some young fishes which I have examined, (haddock, common dab, long rough dab) which have not reached the size at which reproduction begins, and in which the ovaries are very small the larger ova show either no formation of yolked granules in the periphery, or a mere trace. It would seem therefore that the formation of yolk is a convenient and natural character to take in the classification of the eggs.

In dealing with the growth of the intra-ovarian eggs, I shall not deal at length with the earlier stages before the yolk granules begin to be deposited in the cytoplasm. My observations agree on the whole with those of Scharff. Like him, I have found the smallest eggs in the ovary of the haddock (caught on 15th August) along the margin of the lamellae, at their distal ends, or forming digital ingrowths into the stroma. In some cases in the haddock they resembled /

resembled polynucleated masses, hundreds of minute ova, measuring from about 0.014 mm. being crowded together (Fig. 4) with an extremely thin layer of protoplasm and delicate stroma between. The nucleus contains at this stage numerous small nucleoli, not arranged around the periphery of the nucleus, but scattered throughout its substance; and subequal in size. In this haddock there were also present the colloid-looking remains of large retained eggs (a) from the previous spawning some months before; but the minute ova were also found in an immature haddock caught in July, in this case being less abundant and wedged in between the larger ones. In the ova a little larger, ranging about 0.035 mm., the nucleus is surrounded by a zone of deeply stained protoplasm; beyond which is another zone more faintly stained; it contains numerous highly stained nucleoli, one, or more, of which, is frequently, but not always, larger than the rest, and may show a granular or vacuolar appearance in the interior. The nucleoli at this stage are not definitely disposed around the periphery of the nucleus, but are more or less scattered, showing /

showing at the same time a tendency to the former position. This condition was found in the haddock, whiting, lemon sole, plaice, gurnard, dab and eel. In the latter the presence of usually one (Fig. 5), but sometimes two or three very large nucleoli, or germinal spots, highly stained and refringent, and which may be found in ova somewhat larger although then usually of diminished size, is obvious. For example, in an ovum measuring .08 mm, the nucleus of which measured .043 mm, the germinal spot measured .020. In the lemon sole two or three similar large nucleoli were sometimes present; but much smaller relatively than in Anguilla. In the John Dory there are also present one or two large refringent highly stained nucleoli containing a still brighter granule in the interior.

In sections of the ovary of a sole containing mature eggs, and stained with haematoxylin, saffranin and eosin, the small ova, measuring about 0.08 mm, had in many cases two and sometimes three nucleoli larger than the others. In this preparation the intra-nuclear chromatic network is well seen, its appearance /

appearance under a high power (1080 diameters) indicating a coarse network with large meshes and extending equally throughout the nucleus. In the intervals of the network there is a minutely dotted or granular appearance. In one case the chromatic network formed a ring at its periphery connected here and there by strands with the nuclear membrane, and it was more condensed in the centre as if it had contracted and withdrawn from the nuclear membrane. The nucleoli lie free in it.

In eggs a little larger, from about 0.08 mm. to 0.18 a marked feature is the very general disposition of the nucleoli around the periphery of the germinal vesicle which has at this time a distinct membrane, smooth and regular in outline. It is also relatively large and its sharp distinct contour suggests a swollen condition. In the sections of the ovary of the immature haddock referred to above (hardened in picrosulphuric acid and stained with borax-carmin) the ova range in size from 0.08 to 0.16 and possess the following characters. The nucleus is relatively very large and may measure as much as 0.11 in an ovum /

ovum of the largest size stated. It is spherical, has a definite quite smooth regular contour, and the matrix, which has not shrunk from the membrane at any part, is greyish and unstained, and under a high power shows a finely fibrillated or reticulated appearance, although the reticulations cannot well be made out, and it can be seen to be apparently attached to the wall of the nucleus. It extends equally throughout the nucleus from wall to wall. The nucleoli are almost equal in size and are disposed at fairly regular intervals in close contact with the nuclear membrane and frequently flattened against it; they number from six or seven to over twenty, and are relatively very small compared with the germinal vesicle (Fig. 6). They are sometimes surrounded by a clear area, but not at all to the extent that they are at a later stage. The protoplasm around the nucleus is highly stained and shows the usual granular or reticulated appearance. The follicular investment is loose except where two ova are in close contact, and a faint double contour can be made out at /

at the periphery of the protoplasm. This stage differs from the former one, as represented by younger ova adjoining, principally in the larger size of the germinal vesicle, and, specially, in the disposition and character of the nucleoli. In many of the younger ova, in addition to numerous small nucleoli, one much larger is present, and the nucleoli are not all peripherally disposed. There is no appearance of yolk-granules at the margin of the protoplasm.

In the ovaries of an immature common dab, caught on 2nd August, and presenting much the same general appearance, the ova reached a size of 0.162. The nucleus and nucleoli resembled those in the ova of the haddock as a rule, but among the larger eggs the outline of the nucleus was less sharply contoured and somewhat wavy or irregular, and at certain points at the periphery of the protoplasm granules were present. In the ovaries of a gurnard, caught on 10th October, and in which degenerating eggs were present, the ova at this stage showed the granular zone sharply marked off. In the haddock previously mentioned, /

mentioned, which had spawned, the narrow granular zone at the periphery was evident in ova 0.19 mm in diameter; the nucleus and nucleoli had the same character and disposition as in the immature haddock. In an immature whiting, caught in July, the largest ova measured 0.13; the nucleus was large and sharply defined, and the nucleoli peripheral, and no yolk granules were present. In the ovaries of a plaice caught on 20th September, in which the large yolked-eggs for next spawning reached about 0.58 mm, and made up the great mass of the organ, the smaller eggs showed the same condition of the nucleus, the nucleoli being disposed around the margin in close apposition to the membrane. In this preparation the cytoplasm is to a large extent vacuolated, the vacuolation being at the periphery. Calderwood has represented the same condition in the young ova found in the spent ovary of a common dab. In the mature ovary of the sole, caught on 30th April, the smaller eggs showed a similar condition but not to the same extent; the intra-nuclear chromatin network is conspicuous in /

in this preparation.

I have stated that I examined large numbers of the ova in the fresh condition, and I may here describe the appearance of some of the smaller ones. In specimens corresponding to the sizes, I have been dealing with, the nucleus is very prominent, and the nucleoli conspicuous as highly refringent bodies appearing rather larger than in mounted sections. In some cases in which I isolated the nucleus, its surface was knobbed by the projecting nucleoli and a distinct membrane could not be made out. The features were carefully studied ^d in the minute ova of the topknot (Zeugopterus norvegicus). In an egg measuring only 0.06 mm, and magnified 360 diameters, (without a cover glass), one very bright conspicuous nucleolus was observed, which, on focussing downwards, presented optical alternations of light and shade, suggesting an irregular pyramidal form with the apex towards the eye. It was situated towards one side. Within the nucleus a twisted refringent network was obvious, showing alternating bright /

bright and dark points on focussing, and in the cytoplasm near the nucleus two bright points were visible, whether oil droplets or not, it was impossible to say. By careful focussing and illumination, the intra-nuclear network could be seen to extend into the surrounding protoplasm; although the imbedded nucleus gave a faint double contour in optical section, its outer surface (margin) was observed to be irregular and the network could be traced from such irregularities by light and shade, some distance into the protoplasm. A large number of eggs gave the same results.

In eggs a little larger (0.17 mm) the cytoplasm shows very distinctly the division into two zones described by Ransom (62), Scharff (72), Bambeke (7) and others, the inner being darker and showing a coarse reticulated structure, and the outer lighter, with a finer reticulated appearance. The intra-nuclear network is visible, and in the neighbourhood of the nucleus there is one large and several small refringent points. The next size, from about 0.18 mm to 0.22 mm, shows a striking difference in /

in the optical appearance of the ovum. At earlier stages the appearance does not specially indicate, in these fresh eggs, a spherical form; but now this is a marked feature, the nucleus, which is large, measuring (optically) sometimes half the diameter stands out boldly as a translucent globe surrounded by a gradually darkening ring, which obscures its periphery and which shades off into the lighter area towards the margin of the ovum. The dark part of the ring may measure about 0.02 mm. Above the nucleus there are a large number of minute scattered clear globules, varying in size (Fig.7). This condition becomes more obvious in larger eggs, the nucleus becoming quite obscured by the prominent globules which are obviously of an oily character, and vary considerably in size. In an egg 0.6 mm, and therefore one of the largest opaque, the larger oil globules may measure 0.07 mm ~~(Fig.8)~~. In some they are more scattered; but they form a well-defined cluster around the nucleus.

On /

On now examining a section from the same ovary (which contained ripe fully mature eggs) hardened in picro-sulphuric acid, and stained with haematoxylin, saffranin and eosin, the minute ova, about 0.06 mm in diameter, show the usual highly-stained protoplasm around the nucleus, but I was unable to discover the large conspicuous nucleoli in the latter, so prominent a feature in the fresh condition. The nucleus under the same power (^{0.6x}Zeiss, Oc. 4) shows a granular character and is rather deeply stained, and in some cases where retraction has taken place, deeply stained threads can be traced passing to the nuclear wall. Under a higher magnification (Zeiss 4 mm apochr., Oc. 12) the coarse intra-nuclear network becomes obvious, with a finely granular lighter matrix; nucleoli are by no means conspicuous and difficult to discriminate from the nodes in the network; no large nucleolus could be detected. In larger ova, from about 0.16 mm, ~~on~~ the cytoplasm around the nucleus was vacuolated, empty spaces of very various sizes existing between the meshes of the protoplasmic network. The vacuolation in those in which it is least marked is not /

not quite in contact with the nucleus, but a slight distance from it; in eggs a little larger it is close up to the nucleus and extends a considerable distance towards the periphery of the egg.

Examination of the eggs of several other species revealed a similar condition in the perinuclear zone, both in the fresh condition and in sections. In the fresh condition I observed the dark ring of oil-globules around the nucleus in the small eggs, and in those of medium and large size in the following species: grey gurnard, red gurnard, ling, turbot, brill, John Dory; and in my sections the perinuclear vacuolated condition existed in the eggs of the gurnard, Dory, angler and eel as well as in the topknot; sections were not prepared of the others. Both conditions are absent in the fresh eggs and in sections of the following :- plaice, common dab, long rough dab, lemon dab, ~~witch~~, haddock, whiting, bib; and in fresh eggs of the flounder, halibut, saithe. It is therefore present in those in which an oil-globule is a conspicuous feature in the fully mature ovum, and absent /

absent in those in which such an oil-globule is not present. The vacuolated condition is extremely apparent in the eel (Fig. 8). The existence of a zone of oil-globules around the nucleus in young eggs of this species has been pointed out by Owsjannikov (58) and Williamson (83). The latter found them in eggs between 0.18 and 0.27 mm in diameter occupying the whole space between the nucleus and the zona, and in sections the nucleus was seen to be surrounded by a large number of empty spaces. Williamson also mentions the presence of a single large nucleolus in these eggs.

The eel from which my sections were made was 30 inches in length and was caught at the end of May, apparently while on its way to the sea. The ovaries as usual contained a great deal of fat. In the sections this has disappeared as have also the oil-globules in the eggs, which range from about 0.07 mm to 0.25 mm in diameter.

The same condition was observed in the eggs of *Lophius*. In sections of the ovary of this fish hardened /

hardened in picrosulphuric and stained with borax-carminc, the smallest eggs measured 0.039 mm. The protoplasm takes on a high stain, the nucleus has numerous small inconspicuous nucleoli, but not at this stage a large one, as in many other cases. The preparation does not show very well many of the structures, e.g., the yolk. The perinuclear ring of small oil-globules can be made out in some eggs, 0.13 mm in diameter, in the form of scattered vacuoles; it is more obvious in some of the larger eggs (Figs 17, 18). The nucleus in some of the latter (0.33 mm to 0.54 mm in the long diameter) contains one or several deeply-stained rounded bodies or nucleoli, which may differ in number, size and position in different eggs, and usually exhibit zones within them. There is also frequently an irregular aggregation of dark granules in their neighbourhood, but not always. I am not satisfied that this ovary did not undergo degenerative changes before it was prepared; the formation of numerous granules in the nucleus is common as a postmortem change. The larger eggs in

Lophius /

Lophius are ovate or pyramidal, in some sections the nucleus is not spherical or even ovate, but elongated and sometimes almost rod-like, a condition I have also observed in the ovate eggs of the solenette. As will be seen from the figures the arrangement and structure of the ovarian lamellae are peculiar.

~~2. Angler Fish.~~ Since the condition in the developing ovaries of this fish appear not to have been described (/7), although a description of the eggs, as found floating in the sea, and of the embryonic development, has been given by Agassiz^{and} Whitman (2) and by Prince (60) it may be well if I furnish here a brief account of some points which relate to my subject, reserving a more detailed treatment for another place. The ovary consists of a long coiled tube, from six to ten inches in breadth when flattened, and it may reach a length of 36 feet in a fish 4 feet long. It exhibits serpentine gliding or worm-like movements when the living fish is opened; it is flattened and thin-walled, and the germinal epithelium is confined to one surface in a band occupying /

occupying half the circumference. The inner surface of the latter is thrown into digital processes or tubes which project but slightly into the lumen. The arrangement is shown in Figs 17 and 19, in a fish (from which the sections were prepared) caught in February, and which would probably have spawned in June or July. It will be observed that the epithelium between the ovigerous pouches is markedly columnar, and becomes flattened as it passes to the latter. The tissue subjacent to the columnar epithelium is of a peculiar loose mucoid character with numerous branching spaces, not well made out, and richly supplied with dark pigment. On examination with a high power the surface of the columnar epithelium is observed to be ragged from a layer of greyish coagulum, apparently mucus, which covers it. It will be noticed that the protrusion of the ovigerous tube carries with it a stroma composed largely of the peculiar subjacent tissue, and that a pouch is formed at the end which contains a coagulum, greyish and granular under a high power, and which may be possibly /

possibly mucoid. In that case the mucoid slimy substance in which the eggs are imbedded when shed might be derived from this source; it may however be derived from the columnar epithelium. One point to be noted is the excessively large size of one of the eggs in each group; occasionally two large eggs may be present, but one is usually smaller and irregular in shape, and frequently the smaller eggs crowding the pedicle show vacuoles, as from absorption. The large size suggests an extremely abundant supply of pabulum, and it is possible that that is provided by the fluid referred to. Another point to be noted is the relatively thin egg-membrane. The egg of Lophius is, from the morphological point of view, essentially demersal; that is clearly shown by the character of its yolk, by the fact that when freed from its mucoid covering, it sinks in sea water, and by the character of the embryo. The ova are all shed in a connected mass, and hyaline enlargement does not occur. The conjecture of Cunningham (17) that the sheet of jelly containing the eggs is simply formed /

formed by the egg-membranes coalescing is probably erroneous (—).

The early formation of the oily droplets in teleostean eggs which possess an oil-globule in the mature condition is of interest, especially from their appearance around the nucleus before the yolk granules are deposited in the periphery of the protoplasm. They increase in size and flow together into larger droplets as the ovum increases in size, and in pelagic eggs they coalesce at maturation - when the egg undergoes the physical transformation referred to - into usually one large oil-globule.

It is probable that the chemical relationship between the protoplasm and the oleaginous matter is closer than that between the protoplasm and the highly differentiated yolk spherules. In the retrogressive changes which occur in the protoplasm of the mature egg after death, an early feature is the formation of oily-looking globules. It must also be noted that the presence or absence of an oil-globule in pelagic eggs is of little importance as a distinction between species, since closely allied forms may or /

or may not possess it. But it is invariably present in the eggs of some forms and is absorbed with the yolk at the end of the larval stage. It has no special relation to the floating of the egg and probably represents a food substance which is present in another form in the eggs of allied species which do not possess it. This agrees with the view of Bambeke, but not with Prince (61) who seems to look upon them rather as useless matter. They have been shown to be related to the lecithins (35).

The next feature in the growth of the egg is the formation of the yolk which begins to appear as a peripheral zone of granules in eggs of about 0.2 mm, the size varying a little in the eggs of different species (Figs 9,10) . As the egg grows in size the yolk spherules become distinct and their formation extends inwards to the wall of the nucleus in those forms in which a perinuclear zone of oil-globules is absent (Figs 11,14). In species whose eggs are pelagic, the constitution of the yolk spheres is essentially similar, so far as concerns their microscopic appearance. They are of very different sizes /

sizes in the same egg and may range from about 0.02 mm in diameter in the larger eggs of the plaice down to extremely minute granules. The larger spheres appear to be of a vesicular nature, with granular contents, the granules sometimes being aggregated into separate masses (Fig.15). If such spherules are treated with dilute acetic acid the contents rapidly flow together into minute vesicles which coalesce, the granules dissolving and becoming invisible, while the spherule is indicated by an optical ring which unites with other ^{rings} ~~spherules~~ in contact with it. A five per cent solution of acetic acid dissolves the contents instantaneously. In certain species with demersal eggs the yolk spherules are both larger and of different character, being much more solid and granular. In the eggs of the angler they may reach a size of 0.08 mm, and they are markedly granular in character, the whole interior being occupied by solid-looking refringent spheres with an irregular margin, which sometimes appear as angular crystalline bodies of high refraction. In the egg of the John Dory the larger spherules may measure 0.05. /

0.05 mm, and they are full of very dark coloured granules when examined in the fresh state, and they exhibit a ragged margin. In a section mounted and stained the larger spherules occupy a position intermediate between the egg capsule and the germinal vesicle and not therefore, as is usual, towards the periphery. In such sections they are highly stained and appear as conspicuous aggregations or concretions of smaller spherules and have a mulberry-like appearance (Fig. 15).

This difference in the character of yolk spherules in pelagic and some demersal eggs is shown in other ways. Thus the yolk in the latter is as a rule difficult to flatten beneath the cover glass, even when the thicker capsule of such eggs is taken into account, and both in the fresh and preserved condition they have a resistant or hard feeling between the fingers. For the same reason it is not easy to make good sections of them, as with the egg of the Dory.

As the formation of yolk proceeds, the protoplasmic matrix diminishes in amount and ultimately is represented /

represented by a thin film between the spherules which stains very slightly, and is continuous with the thin cortical layer immediately beneath the zona.

In the largest opaque eggs the yolk spherules near the surface are usually larger than those ^afurther in, and it will be seen when we deal with the maturation of the egg that they flow together on the entrance of fluid into the egg in much the same manner as they do when treated with dilute acetic acid on the slide.

In the course of the examination of my sections, I endeavoured to ascertain the presence of a yolk nucleus, or of chromatic bodies in the cytoplasm, without however making this a special inquiry. In many young ova examined in the fresh condition I detected occasionally a highly refringent granule in the cytoplasm having a faintly greenish hue like the nucleoli in the fresh state, but it might well have been a granule or particle of another kind. In stained sections of the eggs I found occasionally two conditions in the cytoplasm of those where the yolk /

yolk had either not begun to form, or where it was represented by a narrow peripheral zone. In some cases a minute highly stained body not larger than a nucleolus was present, the protoplasm around it not being vacuolated or differentiated. The granule was simply imbedded in it. In other cases the condition was different, a larger and less stained or refringent and usually irregularly shaped body was found surrounded by a paler area or cavity; this condition is shown in Fig. 20 which represents an ovum of the plaice 0.22 mm, in diameter. It will be observed that in this case the chromatic body appears to be partly continuous by processes with the protoplasm. In one of the young ova of the eel, previously referred to, there was a prominent stained part, midway between the nucleus and the periphery, which formed an island, so to speak, amidst the protoplasmic network and the empty (oil) spaces which surrounded it. It was connected on all sides with this reticulum, but whether it should be regarded as anything but an undifferentiated part of the protoplasm is uncertain.

I did not discover a cytoplasmic stained body
in /

*Where is that
shown in Fig 20
?*

in close contact with the outer surface of the germinal vesicle; but in young eggs of the haddock measuring 0.12 mm, and slightly larger, and in which yolk had not begun to be formed, I frequently observed the larger nucleolus, to which reference has been made, closely applied to, and flattened against the inner surface of the nuclear membrane. In one or two cases this larger nucleolus appeared as if it were passing through the wall of the nucleus, suggesting the possibility that it was destined to form the yolk nucleus. It appeared in the midst of the optical section of the nuclear membrane.

The most striking appearance related to this subject that came before my notice, was presented by an egg in a section of the ovary of Zoarces. It measured 0.15 mm, eggs of 0.76 mm being present in the same section, but it was obviously shrunk. The condition is represented in Fig. 21 but the coloured bodies in the cytoplasm are shown darker than they should be. The protoplasm generally is highly coloured, the bodies referred to are of a still deeper tint, and the nucleoli are deeply stained and refringent /

refringent. The outline of the nucleus is irregular, and in some cases nucleoli are situated some distance from the others, surrounded by a clear space. In other sections of the same egg on a higher plane a similar appearance is shown, the nucleus disappearing as the plane of the section rises, while the coloured bodies are still present. They are often crescentic but usually irregularly rounded or oblong. Their disposition shows that they are not artefacts in sectioning. In eggs a little smaller the nucleoli are more numerous and disposed in an irregular circle at the periphery of the nucleus, whose outline is also indistinct, and these bodies are not present in the cytoplasm. The interior of the nucleus in both is occupied by a dense coarse reticulum, slightly stained. If these bodies are related to the ~~phenomena of~~ yolk-nuclei, they are exceptionally abundant.

The origin and function of the chromatic bodies in the cytoplasm of young ova is one of great interest. Bambeke (7) has recently described the elimination of chromatic elements from the eggs of Scorpaena and /

and has discussed the various interpretations which have been assigned to the presence of such bodies in the cytoplasm. He represents in a series of figures the appearances he found in sections of the young eggs, which show in most cases the eliminated chromatic substance in the form of threads connected with the chromatin within the nucleus. He had previously given an account of the vitelline body in the demersal eggs of several species (8).

In 1893, Henneguy also published a paper in which he gave an historical account of the vitelline nucleus (37) and described its occurrence in the eggs of a number of osseous fishes, belonging chiefly to the demersal group, especially in Syngnathus, and in the following year Hubbard described it carefully in the eggs of a viviparous American fish (Cymatogaster) in which he found that it continued until the closure of the blastopore (43)

Two things appear significant in connection with the appearance of the chromatic elements in the cytoplasm. One is the fact that it precedes, or is associated /

associated with, the formation of the yolk granules in the peripheral zone to which the chromatic substance migrates. The other is the fact that in certain demersal eggs, in which the yolk spherules are more fully elaborated, and, so to speak physiologically concentrated, the chromatic elements appear to be more numerous than in pelagic eggs in which the yolk is not so condensed. It suggests the close relationship between the two phenomena, and it is noteworthy that many observers speak of two, three, four or more yolk nuclei as present in some eggs. The distinction between chromatic bodies in the cytoplasm and the yolk nucleus is not clear. Scharff in dealing with pelagic eggs describes the formation of buds from the germinal vesicle carrying with them part of the nucleoli which he describes as breaking up to form themselves the yolk spherules; but this view is untenable, from the great difference in their relative sizes.

I /

I now proceed to consider the large opaque eggs shortly before they undergo the translucent enlargement and acquire the peculiar characters that distinguish the mature pelagic ovum. Large numbers, belonging to various species were examined in the fresh state, and also in stained and mounted sections. The egg-membrane when freed from its follicular investment, presents much the same character in them all, consisting of a clear translucent layer that varies in thickness in different species. In demersal eggs it is usually very thick and tough, frequently of more than one layer, and may possess tubercles and other processes. It is very thin in the flounder and the dab, and much thicker in the plaice and cod. The measurements are shown in the Table given later. It is to be noted that up to the period of maturation, the egg-membrane gradually increases in thickness during the later part of intra-ovarian growth. It may also be stated that measurements of the thickness of the egg-membrane in mounted sections cannot always be trusted, owing to the fact that the re-agents /

re-agents act upon it, and may alter it considerably. The addition of a 5 per cent ^{di}solution of acetic acid swells up the egg-membrane instantaneously and softens it so much that the contents break through; a strong solution of salt acts in the same way, but much more slowly. The membrane of plaice eggs may swell to a thickness of .2 mm, many times more than the normal thickness, in a short time.

The egg-membrane presents in different species differences in markings as well as differences of thickness, and these are useful aids in the diagnosis of pelagic eggs found floating in the sea. It is unnecessary for me to detail the various characters of the surface markings. They are usually of two kinds, both of which are generally found together; one consists in the very common presence of wrinkles, formed by anastomosing or convoluted ramifications, which can be shown by careful focussing to be elevations and depressions. This wrinkled condition varies in different species, both in its intensity, grossness or delicacy, and in the patterns formed by /

by the anastomosing branches. It is very distinct in the egg of the plaice. The second condition consists of minute dottings described as pits, dots, etc., and which may be arranged in lines more or less radiately. In the mature egg of the plaice, I have found this dotted appearance to be limited to an extremely thin superficial layer, which may be found spontaneously separating from the outer surface of the subjacent egg-membrane proper (the reticulated membrane) as a delicate but distinct lamella, in specimens which had been softened in water. I have also observed it in carefully rupturing the large mature living eggs, by pressure, as a thin lamina pushed out in lenticular fashion by the yolk oozing from the rupture in the subjacent membrane, and gradually stripped from it as the quantity of yolk increases. Its importance lies in this, that on viewing the periphery of an egg in optical section, the presence of these minute dots in the surface layer of the membrane simulates fine striations passing through the latter, i.e., it gives the appearance of a distinct zona radiata. I am inclined to think that this /

this has sometimes misled observers who have described the existence of a radiated zona in some pelagic eggs.

The surface markings are important in one respect. I have found that both the intensity of the wrinkling and the aggregation of the dots shown on the surface of the largest opaque eggs are considerably diminished in the still larger fully mature translucent eggs; the change being precisely what one would expect to be presented by the patterns on the surface of an elastic spherical shell, which had undergone physical expansion. For example, in the eggs of the tusk (Brosmius brosme) the surface is characterised by the presence of relatively large distinct points; in the opaque egg seven or eight of these were found to lie between two lines, 0.055 mm apart, while there were only two or three, and never more than four, in the same distance in the fully mature egg (Fig. 22). The distension of the reticulum on the egg-membrane of the sole is also shown in Fig. 22.

Another /

Another point in connection with the membrane is the question whether it possesses fine pores through which fluids may pass, such as have frequently been described by observers in connection with the capsule of demersal eggs. The question of a porous zona has been frequently discussed and disputed, but it is unnecessary to enter much into the matter here; a pretty full discussion of the various views held is given by Brock (10). A radial appearance may be seen frequently in the membrane of pelagic eggs examined in optical section, and in cut sections of that of the gurnard, sole, plaice and solenette, in which it is exceptionally well shown (Fig. 23). I have only examined it carefully in the two latter, and it seemed to me that the radiate appearance was more consistent with the view held by many that it is due to fine fibrillations, rather than to the existence of tubes. In most other forms it appears homogeneous, and no fibrillation or radiate appearance can be made out. In the mature expanded egg it disappears. Many facts however show /

show that the membrane is porous in a physical sense.

The question whether there is more than one layer in the egg-membrane of pelagic eggs has frequently been discussed (10,54); Sars found several lamellae in the egg-membrane of the cod (71), but he has not been followed in this view. The superficial lamella in the egg of the plaice has been referred to. In the gurnard's intra-ovarian egg a double layer has been described by Scharff, and this is usually present in demersal eggs; it is very marked in the intra-ovarian egg of the John Dory, but different in character. The egg-membrane is usually regarded as a product of the vitellus, but the precise mode of formation is obscure. The existence of an extremely delicate inner membrane over the vitellus has also been described. It was first described by Ransom (62) in the egg of the stickleback as "a delicate, colorless, translucent, homogeneous membrane", which he called the inner yolk-sac, his outer yolk-sac being what is usually described as the zona radiata; /

radiata; and it has been described by Oellacher (56) in the trout, by Scharff in the gurnard, and by others; but its presence is usually denied. Scharff found it only in sections of hardened specimens of the young eggs of the gurnard. I have been able to observe it distinctly in some cases when dealing with the fresh eggs. Sometimes in carefully rupturing the true or outer membrane, it was revealed as a delicate pellicle, bounding and retaining the protruding yolk (saithe, flounder, haddock) and then bursting and emitting the yolk with a rush on the slightest increase of pressure. At other times, it was observed by the retraction of the yolk from the wall of the zona or outer membrane. I have represented some of the appearances in Fig. 24. Usually it was ruptured with the outer membrane. It is not a mere condensation of the vitelline surface, at all events morphologically, whatever it may be in origin. In cases where a bulla of yolk was protruded through a rupture in the zona, it was easy to observe its distinct character by slightly diminishing the pressure on /

on the cover glass. The elastic distension of the zona thus brought about caused the yolk spherules in the bulla to rush back again within the egg, and a distinct space was left between the margin of the retreating yolk and the wall of the bulla. With a high power the delicate membrane appeared quite homogeneous and gave a faint double contour. Its function may be of importance after the extrusion of the egg, when a perivitelline space is formed between the zona and the vitellus by the imbibition of sea water. It will then intervene between the latter and the cortical protoplasm or periblast. In the living fertilised eggs of the flounder, at an early stage, I was able to trace it, quite distinct from the cortical layer, up over the edge of the segmenting blastodisc, and in some after death, when the protoplasmic mantle began to retract, as it always does, from the zona, the delicate membrane was carried with it and was thrown into wrinkles in the neighbourhood of the blastodisc, the lines converging towards the latter in a radial manner, and passing sometimes /

sometimes over the edge of the blastomeres.

With regard to the yolk spherules in the well-developed opaque eggs, there is little to add to what I have already said. They form the great bulk of the egg (Figs 11, 23).

The changes in the form of the germinal vesicle have already been adverted to. Its position is central, unless in sometimes the later stages, and in sections it usually appears irregularly angular or stellate, the rays passing from its margin being continuous with the protoplasm lying between the yolk spherules. This is well shown in some sections of the ovary of the plaice fixed with five per cent ^{di}solution of nitric acid, and is indicated in Fig. 11. The nuclear membrane in such preparations is frequently indistinct. In other cases, where picrosulphuric acid was used, this appearance is less obvious owing partly to the shrinking of the nuclear substance. At this stage the nucleoli are not nearly so prominent and highly stained as at an earlier period, and they are smaller and less numerous as a rule; they are /

are irregularly arranged round the margin. In the largest opaque eggs in the ovary of a sole, in which mature eggs were present, the nucleoli numbered from five to twelve in a section, and they were of different sizes. In a germinal vesicle, measuring 0.15 mm, the ovum measuring 0.61 mm, the largest nucleolus measured 0.006. In the ovary of a bib, preserved like that of the sole in picrosulphuric acid and containing mature eggs, the nucleoli were much more numerous, numbering frequently over twenty, but they were also smaller. The disposition and appearance of the nucleoli in the solenette at this stage is represented in Fig.23.

I was unable to make out chromatin network or threads in the germinal vesicle of these advanced eggs, with Zeiss' 4 mm apochr. and compensating ocular 12. It is distinct in the smaller eggs lying beside them (Fig 23 a). With the possible exception of the very early stages when it is difficult to discriminate the condition, the nucleoli are at no time of the nature of karyosomes; they lie as distinct /

distinct bodies unconnected with the chromatin threads.

The structure of the large opaque eggs and some features in connection with the germinal vesicle were also studied in fresh specimens. By graduated compression and rupture of the egg-membrane, it was not difficult to isolate the germinal vesicle and to examine it with medium and high powers; and also to ascertain much respecting its physical properties by observing its behaviour when escaping from the aperture and under different conditions. Various appearances of this kind are shown in Fig 25. In the fresh egg of the flounder taken from the living fish and immediately examined in the ovarian fluid, the germinal vesicle on extrusion from the ovum appears as a clear, jelly-like, tenacious semi-fluid substance. It adheres to the outer surface of the egg-membrane, and rolls along it, but clinging to it, in the stream of yolk spherules produced by variations in the pressure on the cover glass. It changes its shape when a stream of yolk impinges against it, but in /

in a slow, flowing fashion, and in passing between two adjacent eggs on the slide; it is obviously soft and tenacious. The germinal vesicle of eggs removed from a fish which has been dead for some hours does not behave in this way. It has lost its adhesiveness, but can be readily made to change its shape (see Fig. 25), and it easily ruptures, which indeed very frequently happened as it escaped from the aperture in the egg-membrane, and a clear syrupy, jelly-like fluid escaped, to which yolk spherules became attached, but which was not adhesive as in the former case. In the opaque eggs of cod, haddock and plaice, which had been kept (in January and February) for a day or more, the contents of the isolated germinal vesicle were much more fluid, running out among the yolk spherules and disappearing. The germinal vesicle both before and after extrusion measures somewhat under one-third of the diameter of the egg. On examination with a high power, delicate fibrillae were sometimes observed passing from the surface among yolk spherules attached to it (Fig. 26). A double /

double contour peripherally was made out with a high power, and in some cases the retraction of the karyoplasm enabled the nuclear membrane to be seen very distinctly. The membrane of the germinal vesicle of a cod's ovum, which contained somewhat watery contents, was left after the latter escaped as a delicate wrinkled film.

The nucleoli are very distinct as a rule, and usually very numerous, as may be seen in Fig 27, which represents the ruptured germinal vesicle from an opaque ovum of the four-bearded rockling. The ovum measured 0.61 mm, the vesicle 0.18 mm, and the nucleoli ranged in size from 0.008 mm to 0.011 mm; they were not circular in contour but more or less angular. In other cases the nucleoli, while still imbedded in the karyoplasm of the unruptured germinal vesicle, had a smoother outline. The above conditions were ascertained in the examination of the opaque eggs in the ovarian fluid. The addition of a one per cent ^{di}solution of acetic acid, or of a ten per cent solution of common salt, clears up the yolk /

yolk, and darkens and renders visible the nucleus; the salt solution acts more slowly.

The ordinary opaque eggs left to soak in a one per cent of common salt for some hours clear up imperfectly, but sufficient to allow the germinal vesicle to be visible as a translucent area in the centre. These re-agents show the dark peri-nuclear ring of oil-globules well in those eggs which possess it; but after a little the globules collect for the most part towards the surface of the egg, owing to the disintegration of the protoplasm between.

When the ova are ruptured in the manner above described, the yolk spherules may be watched issuing in a stream with the almost quite fluid protoplasmic matrix in which they are imbedded; if oil-globules are present they come out in larger coalescing globules. After treatment with strong solution of common salt, the contents come out in a clear syrupy coherent stream; a weak solution of osmic acid, or the application of hot water coagulates the matrix, so that on rupturing the zona the vitelline mass projects, but /

but does not flow, from the aperture. Examination with a moderate power shows the irregular edge composed of projecting fibrillae between which the yolk spherules are attached.

I also investigated as closely as possible the thin cortical layer of protoplasm which everywhere encloses the yolk. It is continuous with the interstitial protoplasm that passes between the yolk spherules, and is, at least in several cases, separated from the egg-membrane, or so-called zona radiata, by the delicate membrane previously described. It may be demonstrated in the fresh opaque egg by careful examination of the periphery of the vitellus, especially when it is retracted from the zona radiata, as sometimes occurs. It is also seen in mounted and stained sections, as shown in Figs 11 and 23, usually as a thin stained line immediately within the zona. In the latter Figure, representing the large opaque egg of the solenette, it is specially well indicated. It is the only part of the pelagic ovum, within the zona radiata, that retains its position /

position and appearance after the transformation at maturation, when it forms the superficial germinal matter in which the blastodisc appears. It can be better studied in fresh eggs than in sections, with respect at all events to the extremely minute, bright, clear granules or vesicles which are imbedded in it, and which I have failed to discover with certainty in stained and mounted sections. The pellicle itself is extremely thin, not more than about 0.015 mm in the large opaque ovum of the flounder, and the granules are crowded much closer together than in the distended mature ovum. I did not measure them in the flounder, but in the mature egg of the gurnard they measured 0.005 mm. They are sub-equal in size and fairly regularly scattered in the protoplasmic pellicle of the mature unfertilised egg, from ten to eighteen being found in a square with a side of 0.10 mm. Kupffer (47) called these bodies in the herring yolk-granules, (Dotterkörner); Ryder (69) and Agassiz and Whitman (1) apply no special name to them, and their significance and fate is not clear. They /

They disappear sometime after fertilisation, but the mode in which they do so has not yet been determined. Optically, they cannot be distinguished from the minute yolk-granules; but they resist dilute acetic acid, and, according to Ryder, ether and alcohol, and they do not stain. In mature eggs which have been dead for days, even after the protoplasmic layer has contracted and partially disintegrated they persist unchanged. They are interesting from the fact that, apart from oil-globules, (when these are present), they are the only definite bodies which can be recognised to remain unchanged within the ovum throughout the period of maturation. The yolk spherules, germinal vesicle and nucleoli all vanish, but the cortical layer with its granules persists.

The large opaque eggs, then, before they begin to undergo the physical changes at maturation, and while still invested by the follicular epithelium, consist of the following parts : - the egg-membrane, or zona radiata, which is relatively thick; an extremely delicate, separable pellicle within, at least /

^ they also resist

least in several cases, which covers, and follows in its retractions, the vitellus; a thin cortical layer of protoplasm with minute shining granules imbedded in it, which is directly continuous with the scanty protoplasmic matrix by which the yolk spheres and granules are surrounded; a germinal vesicle, of large size, with a soft enclosing membrane, continuous with the interstitial matrix, and containing numerous more or less spherical nucleoli, immersed in a somewhat jelly-like substance, and, so far as sections show, no prominent chromatin network.

I have previously referred to the fact that in the mature ovary, a small number of opaque eggs may be found intermediate in size between the great majority of the other opaque eggs, and the fully mature translucent eggs. They present certain transitional features; they are somewhat larger, they are semi-translucent, the yolk spherules are sometimes a little bigger, the germinal vesicle is smaller, and may be excentric, the egg-membrane is thinner, and if oil-globules are present, they begin to appear at the /

the highest part of the ovum (Fig. 3). The changes are transitional and indicate incipient solution of the fabric of the egg, but the egg is still an opaque one with distinct yolk spherules and germinal vesicle. My sections of ripe ovaries do not include any eggs that I can with certainty assign to this stage. In the ovum of the solenette previously referred to (Fig. 23) the cortical layer is relatively thick and the germinal vesicle excentric but not unduly small; besides, the egg is not spherical, and it is not larger than usual. In sections of the ripe ovary of the bib, there are eggs measuring about 0.53 mm, which show an excentric germinal vesicle, measuring 0.09 mm, or even less, with the nucleoli minute and peripheral, and the yolk indicated partly by separated spherules, but mostly by a homogeneous well-stained colloid-looking substance, which has split at intervals. It is represented in Fig. 29. This substance resembles what is seen in the mature eggs in the same section, except that it is more deeply stained and solid-looking; and in other eggs of about the same size, the yolk-spherules have been preserved. But this egg is not /

not larger than the ordinary opaque egg, and the appearance may have been produced artificially. The ovary was not preserved until some hours after the death of the fish; it was hardened in picrosulphuric acid and spirit and stained with haematoxylin, safranin and eosin.

I have found transitional eggs in the fresh ovaries of several species, especially when oviposition is nearing completion, and the ovaries contain chiefly mature translucent eggs, and abundance of ovarian fluid. In the gurnard some of these transitional eggs measured 1.15 mm, the mature ^{eggs} measuring 1.45 mm, and the larger of the opaque ^{ones} measuring 0.9 mm. In the red gurnard, I obtained one which appeared to represent a further stage; it was, however, a translucent egg, measuring 1.41 mm, the largest of the opaque measuring 1 mm, and the fully mature about 1.55 mm; spawning was not far advanced. In this case by careful illumination the faint outlines of a few enlarged spheres could be made out in the interior of the translucent yolk; the oil globule was /

was distinct. The earlier stages of the transition forms were found in several species; but most carefully examined in the flounder, in the living fish and after its death. The fish was practically spent, the ovarian cavities being considerably collapsed, and the contents consisting of translucent eggs and abundant fluid; the sanguineous stroma contained also some opaque eggs. Many of the large eggs showed post-mortem changes, that is to say, they exhibited a faint opacity, and on microscopic examination the cortical protoplasm was found to have shrunk from the egg-membrane, to be greyish or brownish, granular and friable. It is usual for numbers of eggs to fail to be expelled at the end of spawning; their shrunken membranes may be found in the cavity months later.

In searching among these eggs, I found others presenting different characters. Some were in the condition described above as in an early stage of expansion, but larger, and are represented in Fig. 30, as they appeared under the cover glass. They were comparatively soft, and it will be observed that the germinal /

germinal vesicle is small relative to the size of the ovum; four measured as follows 0.344, 0.287, 0.31, 0.365 mm, while the egg was estimated to measure about 1.6 mm; the irregular shape prevented exact measurement. Since the average, fully mature, globular egg of the flounder only measures 0.92mm, it is obvious the compression caused by the cover glass had flattened them considerably. The size of the germinal vesicle was not increased in proportion, inasmuch as in the ordinary, full-sized opaque egg it measures about 0.20 mm. Others of these transitional forms, examined in a watch-glass containing ovarian fluid, measured from 0.72 mm to 0.78 mm; the position of the germinal vesicle was defined in many of them as a clear spot measuring 0.22 mm. An isolated germinal vesicle measured the same; and no nucleoli could be made out in its interior, but it could be seen (Zeiss E. oc. 2) to be very finely granular. The yolk spheres had increased in size, some of them measured 0.19 mm, and many presented a curious appearance, the margin at one part being fretted and ragged, others had the whole edge ragged, and they had, as a rule, fine granules dispersed throughout them, but some were /

were clear vacuoles. A number of the larger opaque eggs were soaked all night in a one per cent solution of common salt, and in the morning some were found to be enlarged, and showing in several cases a clear space between the margin of the vitellus or aggregation of yolk spheres and the zona, and some were scarcely changed. Those which had become enlarged measured about 0.75 mm. On slight pressure being applied under the cover-glass, the yolk spheres ran together forming curious figures; they were obviously droplets of liquid. The germinal vesicle was small, and measured, in one case, 0.165 mm.

I shall now describe some eggs I found in the ovaries of this fish, which presented special features. It has already been mentioned that some of the eggs were dead, with the cortical layer shrunk and more or less central. There were others in which a lenticular blastodisc was formed, sometimes showing irregular pseudo-cleavage and disintegration, sometimes healthy; other eggs showed the periblast in continuous contact with the zona, also apparently healthy. But many full-sized /

full-sized translucent eggs showed other features associated with the presence of vacuoles in their interior, some of which are represented in Fig 32. The one most closely resembling those above described, measured 0.86 mm, and in it the vacuoles - obviously derived from the fusion of yolk spherules - were very large, one measuring 0.27 mm, and of unequal size; their interior sometimes, but not usually, contained a few scattered granules. The egg was not transparent, but translucent, and no clearer spot indicated the position of a germinal vesicle, On focussing down a large, faint, but definite circle was observed (Fig. 32 a). Others, transparent and full-sized, presented the appearance shown in Fig. 32 b, c, d, e, In 32 b, the vesicle b was faintly and finely dotted, and contained a small refringent body. In c, more small vesicles were present at the periphery, some of the largest measuring 0.11 mm; d and e show other appearances. The latter was treated with dilute acetic acid, a few drops of five per cent solution being added to the fluid, and the spot a became dark, while the rest remained unaltered. Many other /

other eggs exhibited similar characters.

The interpretation of all the appearances is not clear. From what has been previously said respecting the yolk spherules, I think there is little doubt the vesicles aggregated at the periphery of the large vesicle represent yolk spherules, but the other features are not easy to explain. They are not the ordinary changes which occur after the death of normal mature eggs in sea water, as I showed by experiment, both for this purpose in those of the flounder, as well as in those of the plaice, to ascertain the post-mortem changes associated with loss of buoyancy. The precise changes undergone by the germinal vesicle at maturation in pelagic eggs are not known (2, 69). The extrusion of polar bodies has been frequently observed (2, 54, 18) both before and after fertilisation (18, 39); although Whitman denies the former (69); but no one has been able to show what becomes of the great bulk of the karyoplasm, or to detect the position of the pronucleus before fertilisation. The morphology of the fully-mature transparent pelagic egg, so far as it can be demonstrated by examination, is /

is simple. The colorless, homogeneous and crystalline vitellus is surrounded by the thin layer of germinal protoplasm, and one or more oil globules may be present beneath the latter (Fig. 33). The whole internal fabric has dissolved. In some cases the germinal matter may be mostly aggregated into the blastodisc before fertilisation. In all cases, when it is formed, it is inferior in the egg floating in the water, and is in relation to the micropyle. The yolk as a rule is quite undivided, forming a single sphere encased by the germinal layer, but in some forms delicate protoplasmic films exist dipping from the periblast into the yolk. In the sole this condition is limited to the part beneath the blastodisc; in the sprat, pilchard, anchovy, and apparently in some eggs belonging to the *Muraenidae* (64) the whole yolk is thus divided into large segments, but the spherules are all dissolved and the yolk remains transparent. The condition indicates that when expansion occurs, part of the interstitial strands of protoplasm between the yolk spherules persists in these /

*There is no
Fig 33.
in Plate.*

these forms, which therefore approximate in this respect to the condition in demersal eggs, as that of the herring.

4. The Physical Changes at Maturation :

I shall now proceed to describe the physical changes in pelagic eggs at maturation, which have hitherto been overlooked; and I may first refer to the statements of other observers bearing upon the point. McIntosh and Prince in their elaborate memoir on the development of Teleosteans (54) do not mention any change; they describe the presence of the periblast and the translucency of the yolk, and say that "the eggs reach maturity by successive strata, a comparatively small proportion of them being ripe and translucent x x x While the ova remain in the body of the fish, they are bathed in a mucilaginous fluid, so that they easily glide over each other, and thus their egress is facilitated" (p. 689). Raffaele, who devoted great attention to pelagic eggs merely says their principal character is that they have a specific gravity less than sea water; he only refers to /

to the imbibition of water to form the perivitelline space after the egg is shed (64 p.4). Ryder (69 p. 459), speaking of the cod, says, "Such partly developed ova, when examined with reflected light, appear whitish instead of a clear, transparent, yellowish tint, such as would be noticed in ripe eggs. This difference in color is due to a change in the character of the plasma enveloping the germinative vesicle, for immediately that the eggs are mature and ready to leave the intra-ovarian cavity, they acquire a remarkable transparency. This must be due to a comparatively sudden blending of the protoplasmic corpuscles of the egg into a homogeneous material very like the white or colorless albumen of a hen's egg." Agassiz and Whitman (2) do not refer to it; Whitman (81) mentions the "clearing up of a pelagic fish egg the moment it comes in contact with water, owing to the dissolving of its opaque granules," but he is describing what occurs in the fully-mature egg after it is shed (apparently of Ctenolabrus, vide 3 p. 11) and the disappearance of the granules in the periblast. Again, Cunningham in a work published last year (17, p. 71 /

p. 71) says "the eggs become more or less clear and transparent when they are ripe x x x the difference between the unripe and the ripe egg is due to a change in the condition of the yolk, which becomes more liquid and less granular"; and similarly describes the condition in his work on the sole (19). Günther does not even mention pelagic eggs in dealing with the reproduction of fishes (30).

I shall first show the change in bulk which occurs by comparing the diameter and volume (ascertained by the usual formula for a sphere) in the larger opaque eggs and in those fully-mature and transparent. It must be noted that there is a slight difference between the diameter of the ripe eggs from the same fish; the mean of a number of measurements, partly founded on Williamson's Observations (84) on Hensen's (31) and my own, is here given. The mean difference in size between the largest opaque and the fully-mature is represented in the accompanying table.

TABLE I. /

T A B L E I.

Species	Opaque		Mature	
	Diameter in mm.	Volume in c.mm.	Diameter in mm.	Volume in c.mm.
Grey Gurnard	0.9	0.3817	1.45	1.5962
Red "	1.0	0.5236	1.55	1.9498
Lesser Weever	0.8	0.268	1.32	1.2042
Mackerel	0.85	0.32155	1.25	1.0226
Dragonet	0.7	0.1795	1.0	0.5236
Cod	0.9	0.3817	1.38	1.3706
Haddock	1.0	0.5236	1.45	1.5962
Bib	0.6	0.1130	0.92	0.4072
Whiting	0.8	0.268	1.2	0.9047
Saithe	0.8	0.268	1.16	0.8172
Ling	0.7	0.1795	1.08	0.6595
Tusk	0.9	0.3817	1.3	1.1503
Halibut	2.5	8.1812	3.8	28.732
L. Rough Dab	0.8	0.268	1.1	0.6969
Turbot	0.7	0.1795	1.02	0.5556
Brill	0.8	0.268	1.3	1.1503
Sailfluke	0.75	0.220	1.13	0.7555
Plaice	1.1 1.21	0.67 0.92758	1.93 1.88	3.764 3.47915
Lemon Dab	0.8	0.268	1.3	1.1503
Witch	0.75	0.2209	1.15	0.7963
Common Dab	0.56	0.0919	0.85	0.3215
Flounder	0.65	0.1437	0.92	0.4072
Sole	0.9	0.3817	1.4	1.4367
Solenette	0.8	0.268	1.2	0.9047

It will be observed that the volume of the fully-ripe transparent eggs is very considerably greater than the opaque before they undergo the transformation. The ratio of increase does not always correspond, a circumstance due in part, no doubt, to the real mean diameter not being obtained; but there are natural differences also. Thus, for example, the eggs of the long rough dab undergo comparatively small increase in bulk at maturation, and this condition is associated with an exceptionally low fecundity. The female of this fish can carry all the eggs shed in one season with ease.

The fact, however, that a three-fold or four-fold increase in volume occurs at this period is, I think it will be admitted, of importance, both from the point of view of embryology, and with respect to its relation to certain phenomena in the life-history of the species. The eggs of some invertebrates are also pelagic, as for example among the free-swimming Copepoda, such as Calanus.

Associated with this expansion of the ovum, there are /

are other changes, the most important being the diminished specific gravity, by reason of which it is enabled to float in the surface layers of the sea, and become a pelagic egg. Before the change occurs, it is a demersal egg, and sinks in sea water. This can readily be shown by cutting out a small portion of a ripe ovary and shaking it in a beaker, when the ripe eggs float to the top, and the few opaque which are shaken out at the same time fall to the bottom. The specific gravity of the ripe eggs of different species varies considerably, but it usually ranges between 1024 and 1027. There is also a variation in the specific gravity of the eggs of the same species, or the same fish, sometimes to a considerable extent, as may be shown by putting a number of them into a vessel of sea water and gradually adding fresh water to reduce the density; some linger on the top, while the great majority have fallen to the bottom. I found a batch of fertilised eggs of the plaice, in the multicelled stage, to possess a density ranging from 1024.8 to 1026.5. There is thus variation in specific gravity as well as in size. It has also been found /

found that the specific gravity may vary during the development of the embryo, the egg becoming heavier and sinking towards the bottom at a certain stage. Some, such as those of Labrax, according to Raffaele (64) leave the surface just before hatching, and others, as in the case of Trachinus vipera, at an earlier period. I have found that the fertilised eggs of the turbot, which possess at first a mean specific gravity of about 1024.8, become rapidly heavier, attaining 1026.6 in a day or two, and 1028 at the end of a week. These changes in the buoyancy of pelagic eggs are ascribed by Raffaele partly to the partial absorption of the yolk, and partly to the absorption of salts by the embryo from the fluid in the perivitelline space. At one time, it was believed that the buoyancy of pelagic eggs was caused by the presence of oil-globules (2) which are, however, as is now well-known, absent in the majority of them.

Another striking change that takes place in the ovum at this period has been previously ^{reversed} ~~adverted~~ to, namely, the crystalline transparency which it assumes, and /

and which is of great service to it as a member of the pelagic plankton, inasmuch as it aids it in escaping detection by the young fishes and predaceous invertebrates which abound in the surface water.

That the transformation from the one condition to the other is rapid, is shown by the rarity of intermediate forms; and the distension of the surface markings of the zona radiata and the diminution of its thickness proves that it takes place not by growth but by the physical expansion of the membrane. The thickness of the zona in the opaque eggs and in the fully distended transparent eggs in several species is given in the accompanying table.

TABLE II. /

T A B L E II.

Species	Opaque	Mature
	in mm	in mm
Com. Gurnard	0.042	0.0261
Red Gurnard	0.044	0.020
Cod	0.04	0.022
Haddock	0.043	0.024
Whiting	0.034	0.012
Bib	0.016	0.01
Ling	0.027	0.016
Four-bearded Rockling	0.02	-
Long Rough Dab	0.017	0.011
Turbot	0.019	0.011
Brill	0.026	0.017
Witch	0.026	0.016
Plaice	0.054	0.025
Lemon Dab	0.036	0.02
Com. Dab	0.017	0.012
Flounder	0.016	0.009
Sole	0.030	0.019
Solenette	0.018	0.01

The /

The zona radiata of the mature pelagic ovum is thus seen to be very delicate. It is however by itself heavier than sea water and sinks. The membrane of demersal eggs is always thicker; for example in the lumpsucker it measures 0.081 mm, in the fifteen-spined stickleback 0.11 mm, and in the pogge 0.13 mm.

The properties of the fully mature expanded pelagic egg, particularly its glassy clearness and its diminished specific gravity, suggest the real cause of the change to be the absorption of watery fluid during the final stage of intra-ovarian development. This is indicated by its softness when compared with the opaque eggs, and by its colour and consistency when placed in hot water, but it may be distinctly shown by determining the relative quantity of water present in the two cases. This has been done for me by Dr Milroy, who found that in the mature pelagic eggs of the plaice, dried to a constant weight at 60°C, at 80°C, at 100°C, and finally at 105°C, the percentage of water was the very high one of 91.86.

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In the opaque eggs treated in the same way, the quantity was found to be only 65.5 per cent.

Certain questions arise in connection with this absorption of watery fluid - whether the fluid consists simply of water or of other substances also; what is its origin; and how is the absorption effected. Dr Milroy states that there is one remarkable circumstance connected with the composition of the mature eggs, namely, that if they are boiled so as to hydrolyse all carbohydrate-like bodies present, no cupric-oxide reducing body is formed, and thus the possibility of mucins, pseudomucins, and carbohydrates being present is excluded. Even excluding the possibility of mucin-like bodies in the vitellus, it is usual for the wall of the ovum to be composed of cellulose or a hemi-cellulose substance. The characteristics above mentioned belong also, as we saw, to the free fluid in the ovarian cavity. It is highly probable, as Hensen surmised, that the free fluid, and no doubt also the fluid that enters the ovum is a secretion of the follicle. When the large opaque egg is removed from the ovary and examined under a low power the follicular investment is usually to be seen in /

in the form of a shaggy covering of considerable toughness. It not uncommonly happened in rupturing eggs by pressure that the follicular epithelium remained intact around the zona, the yolk on escaping making its way between it and the outer surface of the latter. This is more marked in demersal eggs. The follicular epithelium is formed by a multitude of minute irregular hexagonal cells, each with a relatively large nucleus; in the plaice, they measure about 0.011 mm, and, as has been said, they constitute a resistant layer. The general stroma of the lamellae is loose, the connective tissue being scanty^x. Assuming that the follicle secretes the fluid which enters the egg, while the latter is still contained within it, there is the possibility of the secretion obtaining access by means of the micropyle which is then formed (72). But this is improbable and /

^x This is not so in some ovaries, at least, which produce demersal eggs, as in Zeus and Zoarces; and I may here say that the condition of the lamellae in this respect, and of the follicles, as well as the thick, and usually double-layered zona and the character of the yolk enables one to see with tolerable certainty and from a comparatively early stage of the development, whether the eggs are demersal or pelagic. There is no doubt, for instance, that the egg of the John Dory is demersal, although it has not been described except in this paper. With equal certainty, the egg of the halibut and of the eel can be said to be pelagic.

and it seems to me, most likely, that the process is essentially an osmotic one, the fluid secreted by the follicular epithelium passing through the egg membrane. It is to be noted that the free ovarian fluid contains a large amount of chloride of sodium, which is a crystalloid, and it contains also albumins but not mucins or pseudomucins. Our knowledge of normal follicular secretions in higher groups appears to be very slight, or altogether wanting (Hammarsten, 35, p.364). It is possible that the rather high percentage of albumins found in the free ovarial fluid is in part at least derived from the eggs themselves, and represents the osmotic equivalent of watery solution of crystalloid which enters.

The expansion of the ovum from the ingress of fluid bursts the follicular investment, and the egg escapes into the ovarian cavity.

III. The RELATION of MATURATION to certain PHENOMENA in the LIFE-HISTORY of the SPECIES.

It might have been anticipated that the process

I have described as occurring in the pelagic ovum would have certain relations to the life-history of the species in which it occurs. The extraordinary increase of volume, unknown, I believe, in the eggs of any other animals, introduces a physical factor whose influence is augmented by another, namely, that species which produce pelagic eggs must produce their eggs in far greater abundance than is the case with those in which the egg is demersal. This is not *directly* caused by the expansion of the ovum, but merely from the circumstance that a pelagic egg is a wandering egg, and is liable to natural destruction in far greater ratio than the egg which is fixed to the bottom. I have computed the mean annual number of eggs produced by each female of twelve species whose eggs are demersal to be 24,700, while the mean annual number produced by each female with pelagic eggs is 2,388,000, that is to say, about ninety-six times greater. This additional burden, so to speak, thrown upon the female with pelagic eggs, has produced, or is associated with, certain sexual relations of an interesting kind, such as the proportionate abundance and size of the sexes.

Another /

Another consequence of a direct kind is that the yolk in the pelagic egg is diluted by three or four times its volume of a watery and, probably, indifferent fluid. Its nutritive value is therefore diminished, compared with the nutritive value of the demersal egg; this in turn gives rise to a series of results affecting the embryo and the larvae. There is another common difference between the nutritive power of the pelagic and demersal egg. The yolk spherules in the latter case are more solid and concentrated. The limit of concentration in the pelagic egg is doubtless chiefly determined by its main physical function, that of floating.

It is to be noted that the difference in the egg as to its pelagic or demersal character is of little importance as a zoological distinction, since of two closely allied species one may possess a pelagic and the other a demersal egg. Thus the egg of the herring is demersal and that of the sprat pelagic, although the latter species so closely resembles the former that it was for long regarded as the same; it is probable that the eggs of the shads would be buoyant /

buoyant if they were shed in the sea instead of in rivers, for they present features of the pelagic type, and by the nature of the case they cannot float in fresh water. The point alluded to is of interest for it indicates the probability that the imbibition of fluid may occur to a slight extent in demersal eggs also, and that the property has been specially developed in some, and not in others.

The circumstances associated with embryonic and larval life which are dependent upon the dilution of the yolk with watery fluid are various. In the first place it causes the animal pole of the ovum, and the embryo, to be inferior, because the germinal protoplasm and the embryo are heavier than the diluted yolk. It also causes development, caeteris paribus, to be more rapid in the pelagic embryo and larvae than in the demersal, and thus we find that the former hatch more rapidly than the latter; and further, for the same reason, the pelagic embryo is devoid of vitelline circulation. In cases where the yolk consists of solid plates as among Elasmobranchs, or of solid aggregations as in Cottus, the salmon, the angler, and many other demersal forms, the simple arrangement found in pelagic embryos by which the heart is /

is in open communication with the perivitelline sinus around the yolk is insufficient. Comparison of the larvae which issue from demersal and pelagic eggs shows the differences alluded to. Thus the egg of the sea-scorpion is of about the same size as the egg of the plaice; comparing yolk alone it is somewhat less. The former hatches in between three and four weeks, ^{The Larvae} is about 7.5 mm. in length, and has the mouth open. It is in other respects well developed. The egg of the plaice, at the same mean temperature, hatches in about sixteen days; the larvae is 4.1 mm long, and it is destitute of a mouth and otherwise ill developed. Again, the egg of the pogge (Agonus cataphractus) is less than that of the plaice, it takes at least some months in winter to hatch and the larvae is 7 mm in length and well developed.

Thus a group of facts associated with the embryonic and larval stages is explicable by a knowledge that the yolk is diluted in pelagic eggs.

The conditions in adult life arising from the expansion of the ovum in the female are also striking. Thus it is physically impossible for the female /

female producing pelagic eggs to carry at the same time in the mature or expanded state all the eggs that she must shed in any given season, because the volume of the eggs approximates to or exceeds the total volume of the body of the fish. The mean gross bulk of the eggs produced each season by a female in a number of species is as follows : plaice, 1,129.25 cubic centimetres; cod, 6,233.5 c.c; haddock, 718.3 c.c; turbot, 4,723 c.c; halibut, 86,196 c.c. Comparison between the volume of the mature eggs produced and the volume of the body has been made in certain cases. For example, a female cod $32\frac{1}{2}$ inches in length, and having a gross weight of 14 lbs 1 oz had a volume of 6,250 cubic centimetres. The ovaries which were fully matured, and had probably lost some eggs, weighed 3 lbs 13 ozs; and possessed a volume of 1,682 cubic centimetres; so that the volume of the body of the fish minus the ovaries was 4,568 cubic centimetres. It was calculated that the number of eggs present was over 4,550,000, which in the mature expanded condition would be equal to a volume of 6,261 cubic centimetres, or considerably greater than the bulk of the fish. In the flounder, which is the most /

most fecund of sea fishes in proportion to its size, the contrast is still more striking. A large female, examined in February, when the eggs were not fully mature, the largest measuring 0.5 millimetre, weighed 1 lb 15½ oz and was 16½ inches long. The gross volume of the fish was 951 cubic centimetres; the ovaries which weighed 6½ oz., had a volume of 170 cubic centimetres, so that the volume of the body was 781 cubic centimetres. A small portion of the ovary was accurately weighed on a Sartorius balance, and the result of the computation of the eggs was to show that 2,733,860 were present in the ovaries. The volume of these in the mature state would be about 1,114 cubic centimetres, or more than 1¾ pints.

The facts stated explain the gradual spawning of fishes with pelagic eggs, which ripen and are shed in successive lots, a circumstance first described by Earll in the cod (24). In many cases, the female carries in the opaque condition the full number, or almost the full number, of the eggs to be shed during a given season; it is the expansion of the ovum which prolongs /

prolongs the spawning period.

It has also been shown by the writer (62,63) that among fishes with pelagic eggs the females are both more numerous than the males and larger, while among those with demersal eggs the contrary usually prevails. The difference depends for the most part upon the prolonged or short period of oviposition, and on the volume of the eggs to be produced, both closely associated with the expansion of the pelagic ovum at maturation.

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EXPLANATION of FIGURES

- Fig. 1. The nearly mature, demersal, ova of the Lump sucker x 5.
- Fig. 2. The nearly ripe, and ripe eggs of the plaice, showing the difference in size and the vascular plexus on one follicle x 5.
- Fig. 3. Transition ovum from flounder. x 40.
- Fig. 4. Section of edge of a lamella in the ovary of a haddock showing ingrowths of minute ova, Zeiss E.
- Fig. 5. Section of small ovum from ovary of eel - Zeiss A. Comp. Oc. 12.
- Fig. 6. Section of ovum of haddock showing the large globular nucleus and the peripheral disposition of the nucleoli. Zeiss A. Comp. Oc. 12.
- Fig. 7. Fresh ovum of Zeugopterus norvegicus, 0.2 mm, showing the formation of oil globules around the germinal vesicle.
- Fig. 8. Section of ovum of eel showing empty spaces where oil globules were (A Oc. 12).
- Fig. 9. Section of ovary of haddock showing ova of various sizes, and the beginning of yolk formation (A oc. 4).
- Fig. 10. The same. x 200.
- Fig. 11. Section of ova of plaice (A Oc. 4).
- Fig. 12. " " smaller ovum in above x 200.
- Fig. 13. Section of ovum of gurnard x 200.
- Fig. 14. /

- Fig.14. Section of ovary of gurnard (A Oc. 4).
- Fig.15. Yolk spherules a of Dory, b of sole, c of Angler. x about 260.
- Fig.16. Section of ovum of Dory (A. Oc. 4) a the germinal vesicle of same x 260.
- Fig.17. Section of ovary of the Angler x 50.
- Fig.18. Same, showing germinal vesicle of large ovum x 200.
- Fig.19. Same, showing the columnar epithelium and the subjacent pigmented tissue x 200.
- Fig.20. Ovum of plaice, showing vitelline body (A.oc.4)
- Fig.21. Ovum of Viviparous blenny x 200.
- Fig.22. Surface markings on Zona radiata of sole (A) and tusk (B). a of opaque eggs, b of translucent mature eggs.
- Fig.23. Ovum of solenette x 120 a nucleus of young ovum in same section.
- Fig.24. Showing Ransom's inner yolk sac. A ovum of saithe under pressure, B the same with pressure relieved, C. retraction from Zona (a) c cortical layer of protoplasm.
- Fig.25. Showing the germinal vesicle of the opaque eggs pressed under cover-glass; a, g.vesicle.
- Fig.26. Germinal vesicle of lemon sole, showing fibrillations passing among yolk spheres.
- Fig.27. Ruptured germinal vesicle of the four-bearded rockling.
- Fig.29. Large, opaque ovum of bib.
- Fig.30. /

Fig.30. Transition eggs from flounder, under pressure of cover-glass.

Fig.31. Yolk spheres found in such eggs.

Fig.32. Appearances found in large transparent flounder eggs from ovary.



